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CD103$^+$ Dendritic Cells Producing Interleukin-12 in Anticancer Immunosurveillance

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The mechanisms through which tumor antigen-specific T cells are elicited in natural or chemotherapy-induced immunosurveillance have been elusive. In this issue of *Cancer Cell*, two papers by Broz and colleagues and Ruffell and colleagues delineate an important role for a specific dendritic cell subset characterized by CD103 expression, dependence on transcription factors *Batf3* and Irf8, and interleukin-12 production.

Cancers progress in an unrestrained fashion when immunosurveillance fails or when tumor cells either actively suppress the anticancer immune response or successfully hide from recognition by lymphocytes. Specific recognition of tumor antigens by CD8$^+$ cytotoxic T lymphocytes producing a particular interferon-γ (IFN-γ)-centered cytokine pattern (Tc1 cells) is one of the cardinal features of natural or therapy-induced immunosurveillance, and the abundance of such Tc1 cell in the tumor at diagnosis has a positive prognostic impact on major human malignancies (Fridman et al., 2012). Accordingly, the effects of conventional anticancer therapies largely correlate with preexisting Tc1 cell infiltration (Stoll et al., 2014; Zitvogel et al., 2013). Depletion of CD8$^+$ T cells or neutralization or ablation of IFN-γ or its receptor abolishes the beneficial effects of chemotherapy in mouse models (Kroemer et al., 2013).

Accumulating evidence indicates that the local function of T lymphocytes is governed by a panel of distinct myeloid cell subpopulations that modulate the recruitment, antigen-specific activation, and function of T cells. Broz et al. (2014; in this issue of *Cancer Cell*) performed extensive immunophenotypic, functional, and gene expression profiling of myeloid cells contained in mouse tumors to distinguish at least four subtypes: TAM1 (tumor-associated macrophage type 1 cells, CD11c^{lo}CD11b^{hi}MHCII^{hi}), TAM2 (tumor-associated macrophage type 2 cells, CD11c^{hi}CD11b^{lo}MHCII^{lo}), CD11b$^+$ DC1 (dendritic cell type 1 cells), and CD103$^+$ DC2 (dendritic cell type 2 cells). CD103$^+$ DC2 expressed typical DC markers (and hence were CD135(Flt3)$^+$CD117(cKit)$^+$ CD26$^+$) and exhibited distinct transcriptional signatures consistent with enhanced cross-presentation, increased costimulation, and heightened expression of chemokines that may enhance T cell interaction (such as CCL5) (Figure 1). Moreover, CD103$^+$ DC2 were able to maintain a basic endocytic compartment and, consistently, were particularly efficient in stimulating naive tumor antigen-specific T cells in vitro, suggesting that CD103$^+$ DC2 might play an active role in stimulating anticancer immune responses (Broz et al., 2014).

Broz et al. (2014) found that *CD11c-Cre;Irf4$^{fl/fl}$* mice lacked intratumoral CD11b$^+$ DC1, while *Batf3$^{-/-}$* and *Irf8$^{-/-}$* mice lacked CD103$^+$ DC2. Moreover, diphtheria toxin (DT) injection into mice expressing a DT receptor (DTR) transgene controlled by the *Zbtb46* (*zDC*) promoter (*zDC*-DTR mice) only depleted intratumoral CD103$^+$ DC2, not CD11b$^+$ DC1. Cancer cells engineered to express CSF2 (also known as GM-CSF) selectively expanded CD11b$^+$ DC1 within the tumor bed, while malignant cells expressing Flt3L preferentially expanded CD103$^+$ DC2. These data support the idea that the DC subtypes are ontogenetically and functionally distinct (Broz et al., 2014). Tumor growth was accelerated in *Irf8$^{-/-}$* mice but not in *CD11c-Cre;Irf4$^{fl/fl}$* mice, in line with the possibility that only CD103$^+$ DC2 (but not CD11b$^+$ DC1) participate in immunosurveillance as previously suggested (Hildner et al., 2008). Moreover, adoptive transfer of tumor antigen-specific T cells largely failed to reduce tumor growth in DT-treated *zDC*-DTR mice (depleted for CD103$^+$ DC2) that simultaneously received FTY-720 to block T cell egress from lymph nodes (Broz et al., 2014). Altogether, these results suggest a role for intratumoral CD103$^+$ DC2 in priming tumor antigen-specific T cells.

Of note, CD103$^+$ DC2 expressed higher levels of *IL12b* (coding for the IL-12p40 subunit) than any other myeloid subset, yet failed to express immunosuppressive cytokine IL-10, which was expressed by the other subsets (Broz et al., 2014), primarily TAM2 cells (Ruffell et al., 2014; in this issue of *Cancer Cell*). Moreover, this functional dichotomy, which was measured in untreated chemotherapy-naive tumors (Broz et al., 2014), had relevance for the chemotherapeutic response to paclitaxel. In a model of oncogene-induced breast cancer, Ruffell et al. (2014) found that CSF1 neutralization with a monoclonal antibody (mAb) caused selective depletion of TAM1 and TAM2 (but not of CD11b$^+$ DC1 or CD103$^+$ DC2) and improved paclitaxel-induced tumor growth reduction similar to mAb-mediated IL-10 neutralization. Because the combined injection of αCSF1 and αIL-10

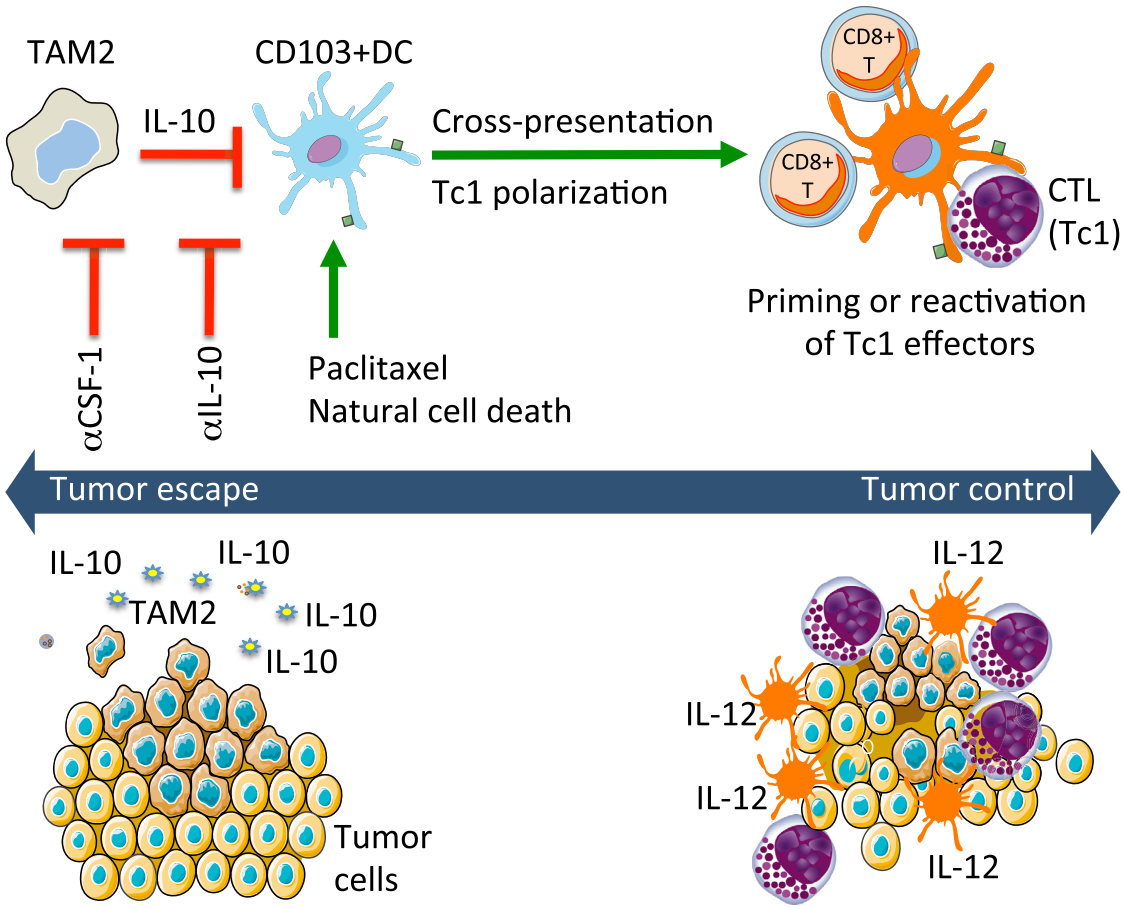

Figure 1. Functionally Important Myeloid Cells in the Tumor Bed
Tumor-associated macrophages type 2 (TAM2), which can be depleted by neutralizing CSF-1 antibodies, produce interleukin-10 (IL-10), which reduces the number and inhibits the function of CD103$^+$ type 2 dendritic cells (DC2). DC2, which can be stimulated by paclitaxel-based chemotherapy, present tumor antigens to CD8$^+$ T cells and produce IL-12 to facilitate the polarization of primed CD8$^+$ T cells to a cytotoxic T cell 1 (Tc1) pattern of cytokine production. These Tc1 cells are the final effectors of anticancer surveillance.

did not confer any further reduction in tumor volume, the therapeutic effects of αCSF1 may be explained entirely by the removal of the IL-10 source. The anticancer effects of αCSF1 and αIL-10 were similar in that they both increased the frequency of CD11b$^+$ DC1 and CD103$^+$ DC2 as well as the expression of *IL12a* (coding for the IL-12p35 subunit) in these populations. Both neutralizing mAbs also increased the expression of *IL12b* (coding for the IL-12p40 subunit) in CD103$^+$ DC2 only, conferring expression of the functional IL-12 p70 heterodimer. Because neutralization of either IL-12p40 or IL-12p70 fully abolished the tumor growth reduction observed with paclitaxel combined with αCSF1 or αIL-10, it appears that IL-12 must act as a positive regulator of therapy-induced immunosurveillance downstream of TAM1/TAM2 depletion and IL-10 neutralization (Ruffell et al., 2014).

Although all the aforementioned results were obtained in mouse models, there is evidence that they may also have some relevance to human cancer. High mRNA levels of *IL12A* (but not *IL12B*) as well as *IRF8* predicted pathological complete responses after paclitaxel-based chemotherapy in breast cancer patients (Ruffell et al., 2014). Similarly, several mRNAs associated with CD103$^+$ DC2 (such as *BATF3*, *CCR7*, *FLT3*, and *KIT*) had a positive prognostic impact on a large collection of human cancers. This also applies to a "CD103$^{+/-}$ ratio" obtained by dividing the signature of genes associated with CD103$^+$ DC2 by those associated with all other myeloid subsets. A high "CD103$^{+/-}$ ratio" predicted improved overall survival in breast cancer, head and neck squamous cancer, and lung adenocarcinoma (Broz et al., 2014).

The aforementioned data support the notion that CD103$^+$ DC2 producing IL-12 play a cardinal role in natural and paclitaxel-induced anticancer immunosurveillance in mouse models of breast cancer as well as human cancer. However, these results contrast with the observation that doxorubicin-induced reduction of EL4 thymoma growth was intact in *IL-12Rb*$^{-/-}$ mice (Ghiringhelli et al., 2009) and that F244 sarcoma cells responded to doxorubicin in *Batf3*$^{-/-}$ hosts (Ma et al., 2013). Unfortunately, no systematic study has addressed whether distinct DC subsets are involved in anticancer immune responses induced by different chemotherapeutics in the context of different tumor types or anatomical localizations. Moreover, there are discrepancies

with regard to the precise localization of DC subsets within the tumor bed. Broz et al. (2014) found that both CD11b$^+$ DC1 and CD103$^+$ DC2 were preferentially located in collagen-rich zones distal to the tumor nodules where TAM1 and TAM2 cells were found. In contrast, Ruffell et al. (2014) report that CD103$^+$ cells were dispersed throughout the tumor stroma in the proximity of macrophages. Although Ruffell et al. (2014) found no change in the localization of CD103$^+$ cells after treatment with paclitaxel and αCSF-1, Ma et al. (2013) found that CD11b$^+$ cells exhibited a selective tropism for dying tumor cells after doxorubicin treatment.

Irrespective of these discrepancies, however, the accumulating evidence suggests that some DC subpopulations can cross-present tumor antigens within the cancer without needing to migrate to lymph nodes. Thus, lymphadenectomy fails to affect the anticancer immune response elicited by anthracycline-based chemotherapy (Ma et al., 2013, 2014). Moreover, direct purification of intratumoral DC subsets yields functional tumor antigen-presenting cells that are able to prime naive T cells in vitro (Broz et al., 2014) and elicit anticancer immune response upon adoptive transfer in vivo (Ma et al., 2013). These results reinforce the idea that the tumor may be considered as a full-blown lymphoid organ, in which all steps of cellular immune responses starting with appropriate presentation of tumor antigens by dendritic cells occur in situ.

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Building through Breaking: The Development of Cancer Neochromosomes

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In this issue of *Cancer Cell*, Garsed and colleagues combine chromosome flow sorting and deep sequencing to characterize the structure of oncogene-containing neochromosomes in liposarcoma and provide evidence that they are generated by a combination of multiple dynamic and destructive processes.

Loss of genomic integrity in cancer has many different manifestations. In this issue of *Cancer Cell*, Garsed et al. (2014) investigate one of the most convoluted products of this genomic instability—the neochromosome, characteristic of well-differentiated/dedifferentiated liposarcoma (WD/DDLPS). The term "neochromosome" describes a marker chromosome whose origin cannot be determined by conventional chromosome banding techniques, which emphasizes their extreme divergence in size and structure from any normal chromosome. By combining chromosome flow sorting and deep sequencing, Garsed et al. (2014) characterize the structure of these remarkably large and highly rearranged structures and propose a model for their genesis and growth. Undergoing multiple rounds of such catastrophic events as chromothripsis, breakage-fusion-bridge cycles, and centromere erosion, their survival is a testament to the power of selection and the ability of tumors to leverage destructive processes for their own benefit.

The heterogeneous collection of malignant tumors of adipose tissue known as liposarcomas constitutes roughly 20% of all sarcomas (Dei Tos, 2014). The most common subtype of liposarcoma, accounting for nearly half of all cases, is the WD/DDLPS, also referred to as atypical lipomatous tumor in some circumstances. Although the higher grade DDLPS is metastatic in roughly 20% of cases, the primary difficulty of this malignancy is associated with local aggressiveness and recurrence. Early cytogenetic studies